

# JOURNAL OF ANIMAL SCIENCE

*The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science*

## **Relationships of testicular iron and ferritin concentrations with testicular weight and sperm production in boars**

T. Wise, D. D. Lunstra, G. A. Rohrer and J. J. Ford

*J Anim Sci* 2003. 81:503-511.

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jas.fass.org/cgi/content/full/81/2/503>



**American Society of Animal Science**

[www.asas.org](http://www.asas.org)

# Relationships of testicular iron and ferritin concentrations with testicular weight and sperm production in boars<sup>1,2</sup>

T. Wise<sup>3</sup>, D. D. Lunstra, G. A. Rohrer, and J. J. Ford

Roman L. Hruska U.S. Meat Animal Research Center, USDA, ARS, Clay Center, NE 68933-0166

**ABSTRACT:** The inverse relationship of testicular size and circulating follicle-stimulating hormone (FSH) concentrations has been documented, and accompanying this relationship is the change in color of the parenchymal tissue of the testes. Large testes (300 to 400 g) are pink to light red and small testes (100 g) are dark maroon with color gradations for weights in between. It was hypothesized that this color most likely represented an iron protein. Chromatographic analysis of testicular tissue indicated that the Fe was associated primarily with ferritin, and immunohistochemistry showed that Leydig cells were the primary location of ferritin storage within the testes. Concentrations of Fe and ferritin were higher in small testes and decreased as testes weight increased ( $P < 0.05$ ). As testicular Fe

concentrations increased, daily sperm production (DSP) and total DSP declined ( $P < 0.05$ ). Genotyping six generations of Meishan  $\times$  White composite boars ( $n = 288$ ) for a quantitative trait locus that is indicative of elevated FSH and small testes in boars indicated that the Meishan genotype had elevated testicular iron concentrations and darker color in conjunction with reduced total DSP ( $P < 0.01$ ). It is not thought the elevated iron concentrations affect testicular weights but are probably a result of elevated FSH and FSH inducement of Fe transport. The storage of Fe in Leydig cells may provide a reservoir of Fe for easy access by Sertoli and germ cells, but still provide a degree of protection to germ cells from ionic iron.

Key Words: Iron, Leydig Cells, Testes

©2003 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2003. 81:503–511

## Introduction

Paradoxically, boars with large testes have lower serum FSH concentrations than boars with smaller testes (Ford et al., 1997; 2001), and Chinese Meishan boars have increased FSH levels and particularly small testes (Wise et al., 1996; Lunstra et al., 1997). It was observed that as boar testes became smaller, the color of the cortex became darker; thus, large testes have a pinkish color and small testes are dark maroon (Wise et al., 1999; Ford et al., 2001). A quantitative trait locus (QTL) for testicular size and FSH concentrations has been localized on the X chromosome (Ford et al., 2001; Rohrer et al., 2001), but the genes responsible for differences

in testicular size have not been identified. The color differences in relation to testicular size were most likely related to iron/iron protein content, and excessive iron can be toxic to sperm and reduce testicular size in humans and rats (Kelly et al., 1984; Lucesoli et al., 1999). Boar testicular size is probably the most important variable in sperm production (Huang and Johnson, 1996), and it has become an economically important trait in an industry that is quickly changing to artificial insemination for breeding purposes.

The initial objective was to identify the biochemical source of the darkening color of the testes associated with testicular size changes and to relate that to other phenotypic changes associated with differences in testicular size. A second objective was to relate the QTL on the X chromosome for elevated testicular size and sperm production with the source of testicular color in a diverse population with large differences in FSH concentrations and testicular weight. Further studies of phenotypic traits associated with small and large testes in swine may lead to the mechanism and gene that has an important affect on testes size.

## Materials and Methods

### Boars

Boars were produced as a resource population for the porcine genomic map as described by Rohrer et al.

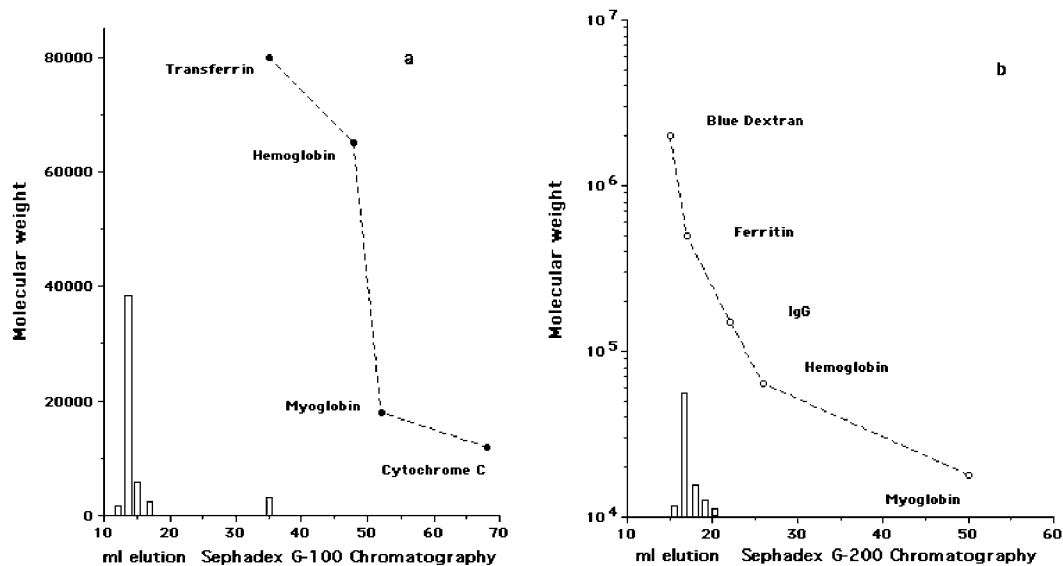
<sup>1</sup>Mention of trade names or commercial products in this article is solely to provide specific information and does not imply recommendation or endorsement by the USDA.

<sup>2</sup>We thank the Swine Operations Personnel for care of the animals; L. Parnell, M. Judy, S. Hassler, and A. Kruger for technical assistance; D. J. Dias for FSH antisera; and USDA/NIDDK for oFSH, pFSH, and FSH antisera.

<sup>3</sup>Correspondence: P.O. Box 166 (phone: 402-762-4185; fax: 402-762-4382; E-mail: wise@email.marc.usda.gov).

Received May 10, 2002.

Accepted August 20, 2002.



**Figure 1.** Chromatographic profiles of testicular homogenates on Sephadex G-100 (a; 15 mm  $\times$  43 cm) indicated a red/brown-colored protein that eluted in the void volume. Subsequent chromatography on Sephadex G-200 (b) indicated that the colored protein eluted with the ferritin standard with a molecular weight in excess of 500,000. Dotted lines represent standards and columns on the x-axis represent eluted proteins measured at  $A_{550}$ .

(1999; 2001) and consisted of  $\frac{3}{4}$  Meishan  $\times$   $\frac{1}{4}$  White composite,  $\frac{3}{4}$  White composite  $\times$   $\frac{1}{4}$  Meishan, and  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite. Boars ( $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite) from generation  $F_4$  (Ford et al., 2001) either were subject to unilateral castration at 1, 10, 56, or 110 d of age or were control boars with both testes. Treatments were to maximize the variation in FSH concentrations and subsequent testicular size. Testes were removed at 220 d of age ( $n = 132$ ). These testes were used to characterize interrelationships of testicular iron concentrations with FSH, ferritin, color, sperm production, and testicular weights. Boars of the  $F_6$  and  $F_7$  generations represented an outcross of the  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite population resulting in  $\frac{1}{4}$  Meishan  $\times$   $\frac{3}{4}$  White composites. Body weights, when the testes were removed for  $F_1$ , BC (backcross of  $\frac{3}{4}$  Meishan and  $\frac{3}{4}$  White composites to produce a  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite),  $F_3$ ,  $F_4$ ,  $F_6$ , and  $F_7$  generations were  $145.5 \pm 6.0$ ,  $128.6 \pm 4.7$ ,  $168.5 \pm 4.1$ ,  $109 \pm 2.8$ ,  $178.6 \pm 4.6$ , and  $127.6 \pm 5.1$  kg, respectively. Boars consisting of 90  $\frac{3}{4}$  boars ( $\frac{3}{4}$  Meishan  $\times$   $\frac{1}{4}$  White composite and  $\frac{3}{4}$  White composite  $\times$   $\frac{1}{4}$  Meishan) and 198  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite boars and representing five generations were genotyped for a QTL residing on the X chromosome for FSH and testicular size (Ford et al., 2001; Rohrer et al., 2001) in which the Meishan allele relates to small testes and high FSH concentrations and the White composite allele for large testes and low FSH. Testes were removed at slaughter or under general anesthesia (Ford et al., 2001). Care and handling of boars complied with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

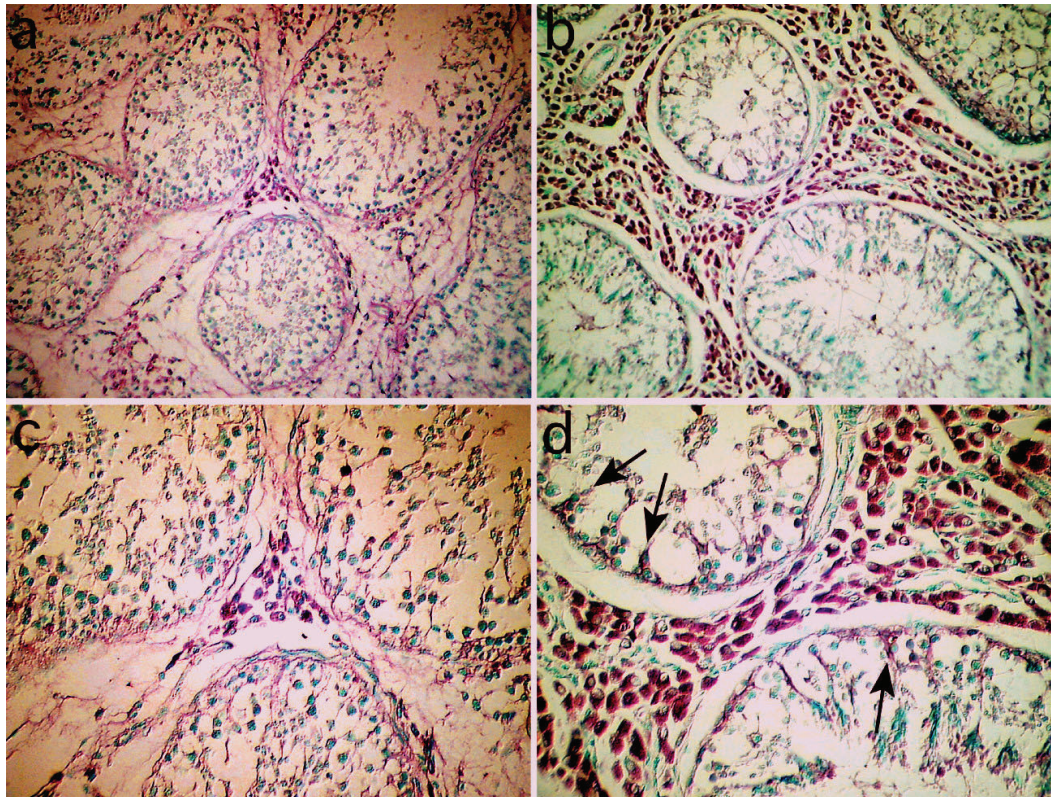
### Assays

Testicular color score was derived by visual color matching with a computer-generated 10-point scale ranging from light pink (1) to dark maroon (10). Light- ( $1.5 \pm 1$ ) and dark-colored testes ( $6.5 \pm 1$ ) were homogenized (10 g/100 mL), centrifuged ( $1,500 \times g$ ), and the supernate analyzed for hemoglobin. Hemoglobin in supernates of testicular homogenates was measured colorimetrically at 540 nm by the cyanmethemoglobin assay procedure (Sigma Diagnostic Products #525, Sigma-Aldrich Chemicals, St. Louis, MO). Ferritin was immunologically measured with the Tina-quant assay for human ferritin (Tiffany and Burtis, 1987; Palencia-Dominguez et al., 1997) supplied by Boehringer Mannheim Corp. (Indianapolis, IN). Iron, copper, and zinc content of the testicular tissue were measured by atomic absorption spectroscopy (Perkin-Elmer 1100, Overland Park, KS). Circulating concentrations of FSH were measured by established radioimmunoassays (Ford et al., 1997; 2001). Boars were bled at least twice, and generally three times, for FSH concentrations and averaged for statistical analysis. Sensitivity of the FSH assay was 40 ng/mL, and the interassay coefficient of variation for four pools of varying concentrations ranged from 11 to 18%. Daily and total sperm production were calculated by homogenizing testes (polytron homogenization of testes in 25 mM Tris, 0.25 M sucrose, 1 mM EDTA, and 0.1% triton X for 20 s) and subsequent counting of intact sperm via hemacytometer (Amann and Almquist, 1961; Rathje et al., 1995).

### Iron Protein Analysis

To identify the protein source of dark color, testes were initially homogenized in 25 mM Tris, 0.25 M su-





**Figure 2.** Immunohistochemical staining for ferritin in large testes (a, c) and small testes (b, d) at 20 $\times$  and 40 $\times$  magnification (a, b and c, d, respectively). Sections were counterstained with fast green. Primary staining for ferritin is found in the Leydig cells (red), but a faint outline of staining is found around the circumference of the tubule. Occasional staining of structures was detected extending into the tubule from the basement membrane and most likely represents Sertoli cell staining for ferritin (arrows).

crose, 1 mM EDTA, and 0.1% triton X, centrifuged to remove particulate material (Sorvall RC-5, 40,000  $\times$  g for 60 min), and aliquots of supernatant submitted to Sephadex chromatography for molecular weight determinations (Figure 1). For final chromatographic identification, porcine testicular ferritin was isolated by the method of Worwood et al. (1975) and May and Fish (1977). Testicular homogenates were heated to 75°C for 10 min (Worwood et al., 1975) in 40% methanol (Cham et al., 1985) and finally centrifuged. The high temperature of incubation and methanol step precipitates the majority of proteins, but ferritin is unaffected and stays in solution. Samples were dialyzed against 0.25 mM Tris and 0.25 M sucrose and subjected to Sephadex G-100 and G-200 chromatography. Molecular weight standards were purchased from Sigma-Aldrich Chemicals. Column chromatography elution profiles were monitored at A550 for presence of hemoproteins. Protein content of homogenate was determined by a modified Lowry procedure (Markwell et al., 1978).

### Histology

To identify cell types (Leydig or Sertoli) that would indicate presence of Fe color proteins and to compare differences in large and small testes, testicular tissue

(2 mm<sup>2</sup>; from  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White cross boars, 220 d of age) was fixed in 4% paraformaldehyde, embedded in paraffin, sectioned (5  $\mu$ m), and incubated with anti-ferritin (Fitzgerald, Concord, MA). Antibody binding was visualized with the avidin-biotin immunoperoxidase system (Vectastain Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA) with Nova red as the chromogen. Slides were lightly counterstained with Fast Green to better visualize structural components. Non-specific binding was blocked with 5% goat serum for 1 h. Testicular sections without the primary antibody were not stained and were used as the immunohistochemical control. Histochemical location of iron was by the ferrocyanide method of Perls (1867).

The percentage of Leydig cells within the testes was determined by morphometry on 25% of boars with the largest and 25% with the smallest testes within each treatment group at 220 d of age (generation F<sub>4</sub>; n = 58). Each testis was cut in half longitudinally, and three samples (each 6 mm<sup>3</sup>, weighing approximately 0.2 g) of parenchymal tissue from the central portion were excised for glutaraldehyde (3%) fixation and embedding in plastic for structural evaluation (Lunstra et al., 1986; Rathje et al., 1995). Serial 1- $\mu$ m sections were cut with glass knives from each of three plastic blocks per testis, mounted on glass slides overnight at 60°C, and stained

with 1% periodic acid, 1% toluidine blue, and 0.5% basic fuchsin solutions (Rathje et al., 1995). Interstitial areas were evaluated at 400 $\times$  magnification to obtain Leydig cell size (approximately 300 Leydig cells per testis were measured to obtain cell size) and area percentages occupied by Leydig cells, myoid cells, and vascular structures (lymphatic and blood vessels), using the morphometric procedures described by Rathje et al. (1995) and Okwun et al. (1996).

### Statistical Analysis

Relationships of Fe, ferritin, FSH concentrations, testicular weight, color, and sperm production were evaluated using the GLM procedure ( $F_4$  generation of  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite) (Data Desk<sup>4</sup>, 1994) with the model of fixed effects of sire, testicular iron concentration, and treatment (time of unilateral castration or control with both testes) as a covariate. In the analysis for FSH relationships, FSH concentrations were log transformed to produce homogenous variances. Correlation coefficients were calculated to provide the strength of relationships (linear) between testicular iron concentrations and testicular weight, color and FSH levels. The GLM model for genotype differences included sire, breeding generation, and genotype and the appropriate interactions. Differences between phenotypic variables represented by White composite or Meishan genotypes were tested by Scheff's procedure.

## Results

### Ferritin Identification

Although the supernatant of dark homogenized testes was distinctly more red than the supernatant from the light-colored testes, there were no differences in hemoglobin concentrations (light =  $14.3 \pm 1.1$ , dark =  $14.6 \pm 0.7$  mg/g testes). Thus, blood flow per gram of testes was not likely altered, and the differences in testicular color and testes size were not linked to hemoglobin (red blood count numbers) content of the testes.

Chromatographic elution profiles of small and large testes indicated the testicular protein of color came out in the void volume ( $>100,000$  molecular weight) and was not hemoglobin or a low molecular weight protein (Figure 1a). Analysis of testicular extracts with Sephadex G-200 chromatography indicated the protein was most likely ferritin, the iron storage protein (Figure 1b). Homogenates from small testes had more red/brown protein, which was clearly visible as the protein migrated through the chromatography column and in the eluate collection tubes. High temperature incubation (70°C) and methanolic precipitation of testicular homogenates, which were also submitted to Sephadex G-200 chromatography, identified the color protein had properties consistent with ferritin. Subsequent immunoassay of Sephadex G-200 column fractions further supported the identification of the protein as ferritin (data not shown).

### Testicular Histology

Initially, testicular sections representing small and large testes were stained for Fe (Perls Prussian blue stain), and paradoxically, all slides had little or no staining, even though the dark color was clearly visible without magnification on histological sections at 10, 25, and 50  $\mu$ m of thickness. Immunochemistry analysis of ferritin in testicular tissue from small and large testes indicated that ferritin was primarily located in Leydig cells with a light staining at the peripheral edge of the tubules and in Sertoli cells (Figure 2a,b,c,d).

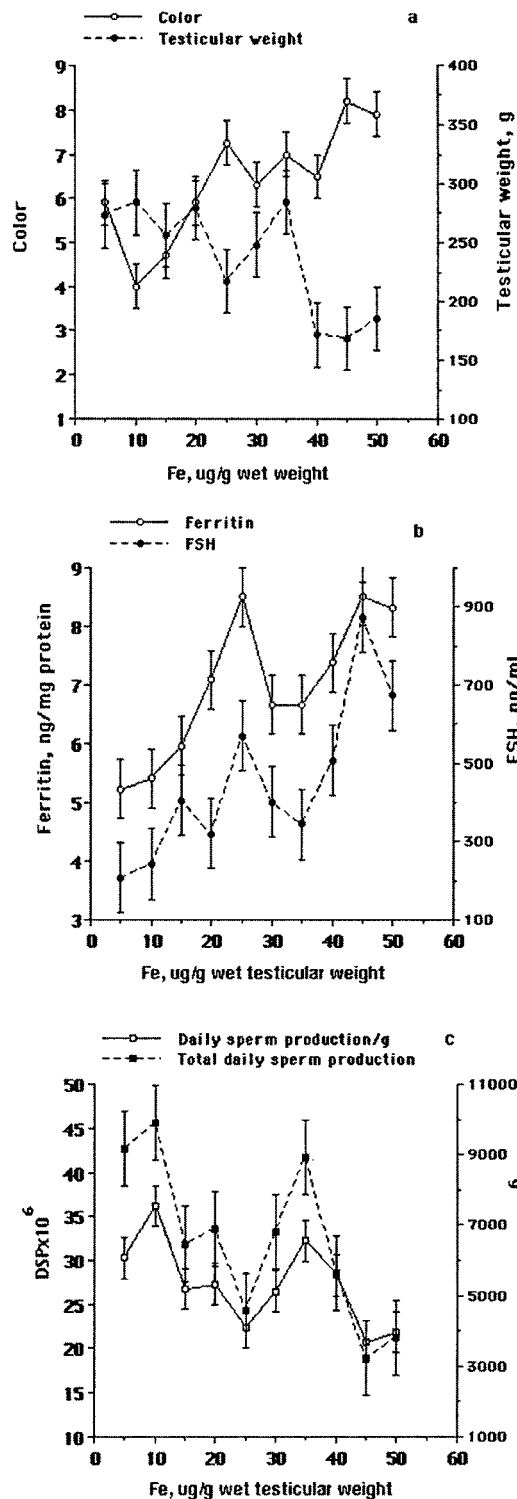
### Testicular Fe, Ferritin, Color Relationships

Analysis of testicular Fe relationships was initiated on 132  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite boars ( $F_4$  generation of inter se mating of  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite parents). Hemicastration of 95 boars reduces feedback effects of inhibin and testicular steroids on the pituitary, results in elevated gonatropins, and was predicted to produce considerable variation in testicular weight and size. Testicular color, Fe, FSH concentrations, daily sperm production (DSP)/g and total DSP per testis (DSP  $\times$  testis weight) were not different between control boars with two testes (one testis used for comparison purposes) and hemicastrates. The lack of any differences between control and hemicastrates allowed responses in relation to Fe concentrations to be pooled for analysis. Testicular color became darker red with increases in Fe concentration (Figure 3a; group effect  $r = 0.50$ ;  $P < 0.01$ ). Testicular weight declined with increases in Fe concentration (Figure 3a; group effect  $r = -0.20$ ;  $P < 0.01$ ) and was inversely related to FSH concentrations. No relationships were noted between testicular weight and testicular Cu or Zn concentrations (data not shown). FSH concentrations increased with elevations in testicular Fe concentrations (Figure 3b; group effect  $r = 0.38$ ;  $P < 0.01$ ). Testicular ferritin concentrations also increased coordinately with FSH and Fe levels (Figure 3b). Sperm production per gram of testes and total DSP declined with increases in testicular Fe concentrations (Figure 3c; group effect;  $P < 0.05$ ). The relationship of testicular weight and percentage of Leydig cells occupied within the testes indicates that as testicular weight declines, Leydig cell volume increases within the testes (Figure 4a;  $r = -0.68$ ,  $P < 0.01$ ). In further support of testicular iron and darkening of color being associated with Leydig cells, Figure 4b,c shows that the percentage of Leydig cells within the testes increases with Fe concentrations ( $r = 0.39$ ;  $P < 0.01$ ) and color of the parenchyma ( $r = 0.74$ ;  $P < 0.01$ ).

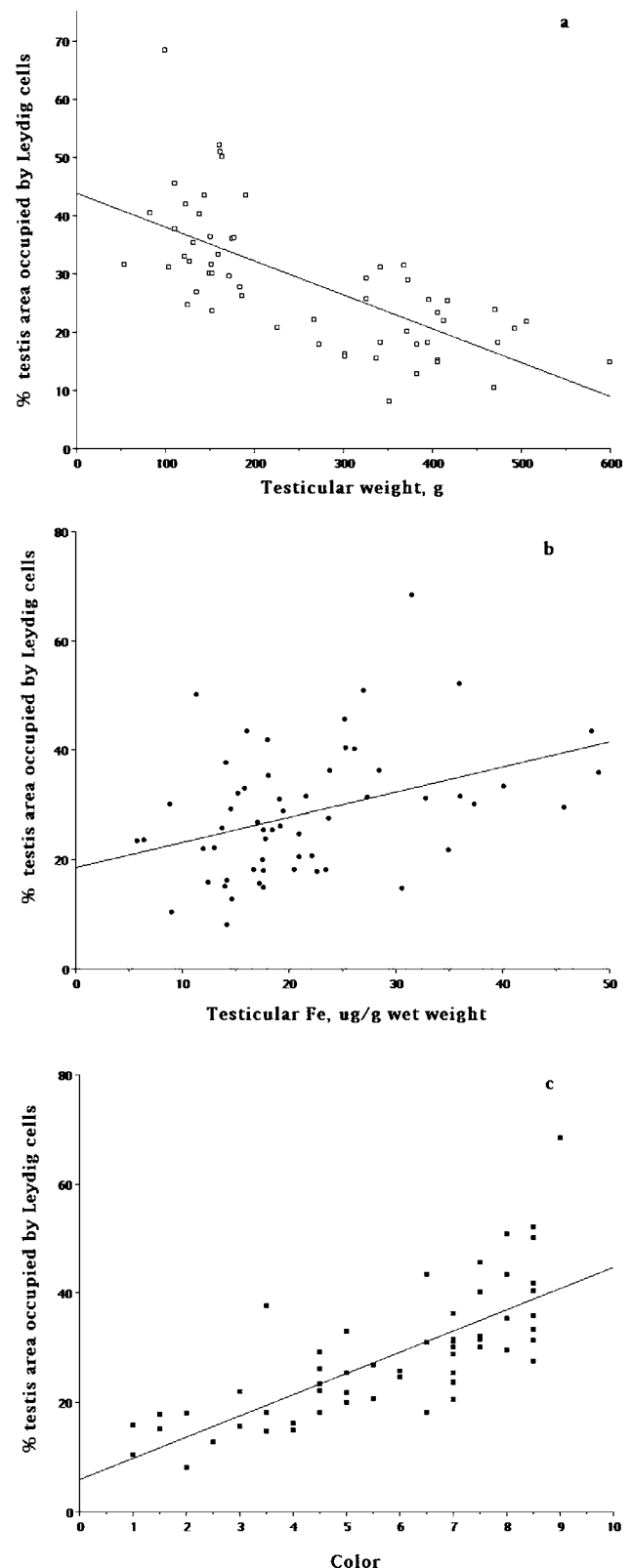
### X Chromosome Quantitative Trait Locus Genotype Analysis

Boars consisting of 90  $\frac{3}{4}$  boars ( $\frac{3}{4}$  Meishan  $\times$   $\frac{1}{4}$  White composite and  $\frac{3}{4}$  White composite  $\times$   $\frac{1}{4}$  Meishan) and 198  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composite boars representing five generations were divided into genotypes for Meis-

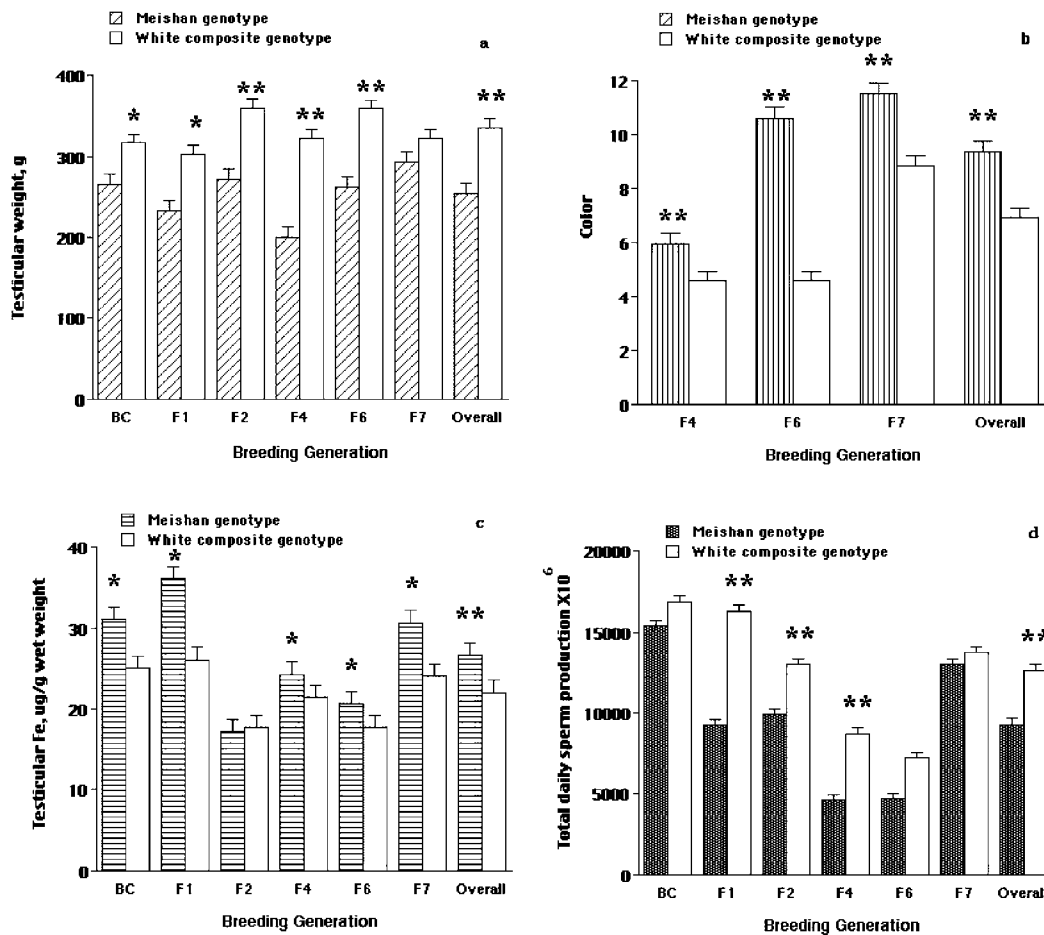




**Figure 3.** Relationships of testicular color and testicular weight (a), testicular ferritin concentrations and circulating follicle-stimulating hormone (FSH) concentrations (b), and sperm production per gram of testes and total daily sperm production (c) with Fe concentrations of the testis. Testicular color increased (group effect of changes over Fe levels;  $P < 0.01$ ) and testicular weight declined (group effect of changes over Fe levels;  $P < 0.01$ ) with increases of testicular Fe (a). Testicular ferritin concentrations and plasma FSH concentrations increased with testicular Fe concentrations (b; group effect of changes over Fe levels;  $P < 0.01$ ). Sperm production per gram of testes and total daily sperm production declined with increased concentrations of Fe (c; group effect of changes over Fe levels;  $P < 0.05$ ).



**Figure 4.** Relationships of percentage of Leydig cells, as determined by morphometric analysis (scatter gram,  $n = 58$ ), within the testis with testicular weight (a), testicular Fe concentrations (b), and color of testicular parenchyma (c). The correlation coefficient for the relationship between percentage of Leydig cells and testicular weight, Fe concentrations, and color was  $-0.68$ ,  $0.39$ , and  $0.75$ , respectively. Color scale (c) ranged from 1 (light pink) to 10 (dark maroon). Lines on figures represent best-fit linear regression lines.



**Figure 5.** Results of genotyping for a quantitative trait locus on the X chromosome for testes weight and follicle-stimulating hormone concentrations represented by a Meishan allele and White composite allele indicated that increased testicular weight was associated with the White composite allele ( $P < 0.01$ ; a) and increased testicular color and Fe with the Meishan allele ( $P < 0.01$ ; b, c). Total daily sperm production was significantly increased in boars with the White composite allele ( $P < 0.01$ ; d). Generations F<sub>1</sub> to F<sub>4</sub> represent generations of  $\frac{1}{2}$  Meishan  $\times$   $\frac{1}{2}$  White composites; generation BC were  $\frac{3}{4}$  Meishan  $\times$   $\frac{1}{4}$  White composites and  $\frac{3}{4}$  White composite  $\times$   $\frac{1}{4}$  Meishan, whereas generations F<sub>6</sub> to F<sub>7</sub> were  $\frac{3}{4}$  White composite  $\times$   $\frac{1}{4}$  Meishan. Stars signify differences between alleles (\* $P < 0.05$ ; \*\* $P < 0.01$ ).

han or White composite characteristics associated with the QTL on the X chromosome for testes weight and FSH concentrations. There were no differences between  $\frac{1}{2} \times \frac{1}{2}$  or  $\frac{3}{4} \times \frac{1}{4}$  boars in relation to testes weight or FSH concentrations and thus were combined for the genotype analysis. Boars possessing the Meishan QTL genotype had approximately twice the FSH concentrations as boars genotyping for White composite QTL. Boars with the Meishan genotype had smaller testes, elevated Fe concentrations, and darker color than boars possessing the White composite genotype (Figure 5). Ferritin concentrations were not different between genotypes (F<sub>4</sub> generation of  $\frac{1}{2} \times \frac{1}{2}$  boars only assayed for ferritin; Meishan =  $6.9 \pm 0.4$  vs White composite =  $7.0 \pm 0.4$  ng/mg protein;  $n = 134$ ). Sperm production per gram of tissue was similar between the two genotypes (Meishan =  $25.3 \pm 1.8$  vs White composite =  $26.1 \pm 1.8 \times 10^6$  DSP/g testis), but the White composite genotype had considerably greater total DSP (DSP  $\times$  testicular

weight) due to the greater testicular weight (Figure 5a;  $P < 0.01$ ). Relationships between FSH, total DSP, testes weight, and ferritin with testicular Fe concentrations show an inflection point around 25 to 35  $\mu$ g of Fe, resulting in a biphasic response when plotted against testicular Fe concentrations (Figure 3). A frequency analysis by genotype revealed that White composite boars were primarily represented by less than 25 to 35  $\mu$ g of Fe, and the Meishan genotype by greater than 25 to 35  $\mu$ g of Fe, thus providing further evidence of phenotypic differences that can be identified by genotyping with markers on the X chromosome.

## Discussion

The darkening of the parenchyma from pink to dark maroon as testes weight decreased most likely indicated that the effect was from a protein with considerable iron content. Greater concentrations of LH increase

blood flow to the testes, and boars with small testes have increased FSH and LH. If testicular weight decreased and testicular blood flow remained the same, then considerably increased numbers of red blood cells may be found in the smaller testes leading to a darkening of the parenchyma. The opposite would be true for larger testes resulting in lighter colored testes. No differences in hemoglobin content were detected within dark- or light-colored testicular homogenates. Darkening of the parenchyma as testicular size declines was primarily associated with accumulation of Fe in Leydig cells, although the mechanism, associated function or possible advantage/disadvantage, is not clearly evident. In association with the increase in levels of testicular Fe is the accumulation of ferritin, where Fe is safely stored within Leydig cells. The lack of histological staining for Fe probably indicated that a combination of the histochemical stain was not sensitive enough to detect the tissue Fe or the Fe as bound to ferritin was unavailable to the stain reaction. The Fe is bound in the center of the ferritin molecule and is probably inaccessible to ferrocyanide color reaction (Harrison and Arosio, 1996). Dark-colored testes had more intense staining for ferritin in the Leydig cells than light-colored testes, due largely to the increased numbers and size of Leydig cells found in small testes (Figure 2c,d). Unlike the liver, gene expression of transferrin and its membrane receptor in Sertoli cells is not controlled by Fe concentrations, but by FSH (Chaudhary et al., 1996; Chaudhary and Skinner, 1999) and other endocrine factors (Davis and Czech, 1986; Huggenvik et al., 1987). Boars with small dark testes have increased FSH concentrations (Ford et al., 2001). Little is known about ferritin, the Fe storage protein (Carreau et al., 1994), even though it has been identified in the testes (Mazur and Shorr, 1950). Iron (due to its cellular toxicity in ionic form) is bound to transferrin as a transport protein. The Fe-transferrin complex is internalized through a transferrin membrane receptor and, after internalization, dissociates the Fe to cellular Fe storage proteins until further use (Wauben-Penris et al., 1988; Harrison and Arosio, 1996). Studies of Fe transport into the testes via the transferrin receptor have focused primarily on the Sertoli cell function (Skinner and Griswold, 1982; Toebosch et al., 1987), but the Leydig cell through its storage of Fe via ferritin may also play a role in Fe homeostasis and may be a primary source of Fe for Sertoli transport to developing sperm. Each ferritin molecule can hold a tremendous amount of Fe (23% of ferritin's dry weight can be Fe) (Farrant, 1954), up to 4,500 atoms of Fe per ferritin molecule (Harrison et al., 1980; Andrews et al., 1988). Thus, large changes in Fe in tissue may be evident without altering cellular ferritin concentrations. Iron is stored as ferrihydrite (FeOOH) (Iancu, 1992) within a large protein core (molecular weight = 450,000 to 500,000), thus through its inaccessibility of Fe within the molecule, ferritin provides protection to the cell and tissue from ionic Fe. Storage of Fe in Leydig cells also provides an extra

layer of protection to germinal cells and still maintains easy availability of Fe to Sertoli and germ cells.

Although there is limited information on the mechanism of control of the testicular transferrin receptor, Roberts and Griswold (1990) reported that FSH increased transferrin receptor messenger RNA levels in testes of hypophysectomized rats, but had no effect on receptor levels in Sertoli cell cultures. Elevated FSH can also increase mRNA for transferrin (Huggenvik et al., 1987; Chaudhary and Skinner, 1999). If increased FSH concentrations in boars induce extra-testicular Fe transport via the transferrin-Fe exchange system, then this Fe must be safely stored. Boars with small testes have elevated LH concentrations in conjunction with the increased FSH levels (Wise et al., 2000; Ford et al., 2001). Elevated LH can also increase c-myc in the Leydig cells (Lin et al., 1988; Hall et al., 1991), where most of the immunohistochemical staining for ferritin occurs. The oncogene c-myc has a role of coordinately regulating genes controlling intracellular Fe concentrations (Lim and Hwang, 1995; Wu et al., 1999). The mechanism by which smaller testes have increased Fe concentrations has not been elucidated, and may be the result of other Fe transport systems, such as divalent metal transporter-1 (Picard et al., 2000; Burdo et al., 2001), rather than the transferrin receptor/transport system.

Human males with  $\beta$ -thalassaemia major have decreased pituitary function from Fe overload, and serum ferritin is highly correlated with the presence of hypogonadism (Papadimas et al., 1996). Excessive Fe is destructive to testicular function and spermatogenesis (Merker et al., 1996; Lucasoli et al., 1999), and smaller testes and reduced sperm production may be related to the elevated Fe concentrations. Even though the demands for Fe may be greatest for sperm production, most of the ferritin and Fe seem to be stored in the Leydig cells, which in itself may provide further protection to the developing gamete within the tubules surrounded by Sertoli cells. This study also shows that the elevated ferritin and Fe concentrations in Leydig cells do not seem to affect the viability of Leydig cells in small testes in that there are probably more and larger Leydig cells/g of testes in small testes than large testes. Leydig cell volume is three times greater in Meishan testes as compared to White composite (Lunstra et al., 1997), and boars with the Meishan QTL for testicular size had larger and more numerous Leydig cells. Large testes have more total numbers of Leydig cells due to the weight/volume of the testes is three to four times that of small testes. Thus, some mechanism to balance available Fe concentrations is probably operational (large testes with low levels of Fe and many Leydig cells vs small testes with high levels of Fe and lower total numbers of Leydig cells per testes). Differences in the number and size of Leydig and Sertoli cells in large and small testes may relate to the paracrine interrelationship between Sertoli cells and Leydig cells (Russell et al., 2001).



There are boars that genotype as the White composite QTL and have small testes, just as there are boars that genotype as the Meishan QTL and have large testes. This emphasizes that testicular size and total DSP is a multigene trait, and other genes have effects on testicular size in conjunction with prominent effects from the X chromosome (Rohrer et al., 2001). Classification of genotype as a Meishan or White composite was still strongly related to high FSH concentrations and small testes even after two generations of outcrossing with White composite boars (resulting in  $\frac{3}{4}$  White composite, generations F<sub>6</sub> and F<sub>7</sub>).

## Implications

The differences noted in testicular weight, iron content, and total daily sperm production by genotyping for markers on the X chromosome may indicate a major gene(s) effect, which, when identified, will allow early selection of boars with a greater capacity for sperm production. As swine production has become more consolidated and vertically integrated, swine breeding has shifted to use of boar studs to provide sperm for artificial insemination. Early identification of poor and superior boars for sperm production could become an important economic issue for more efficient and profitable use of boar studs.

## Literature Cited

- Amann, R. P., and J. O. Almquist. 1961. Reproductive capacity of dairy bulls. I. Techniques for direct measurement of gonadal and extra-gonadal sperm reserves. *J. Dairy Sci.* 44:1537–1545.
- Andrews, S. C., M. C. Brady, A. Treffry, J. M. Williams, S. Mann, M. I. Cleton, W. de Bruijn, and P. M. Harrison. 1988. Studies on haemosiderin and ferritin from iron-loaded rat liver. *Biol. Metab.* 1:33–42.
- Burdo, J. R., S. L. Menzies, I. A. Simpson, L. M. Garrick, M. D. Garrick, K. G. Dolan, D. J. Haile, J. L. Beard, and J. R. Connor. 2001. Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. *J. Neurosci. Res.* 66:1198–1207.
- Carreau, S., P. Foucault, and M. A. Drosowsky. 1994. La cellule de Sertoli. Aspects fonctionnels compares chez le rat, le porc et l'homme. [Sertoli cells. Functional aspects compared in rats, pigs and man]. *Ann. Endocrinol. (Paris)* 55:203–220.
- Cham, B. E., H. P. Roeser, A. Nikles, and K. Ridgway. 1985. A procedure for the purification of ferritin from human liver by heating a methanol-treated homogenate. *Anal. Biochem.* 151:561–565.
- Chaudhary, J., and M. K. Skinner. 1999. E-box and cyclic adenosine monophosphate response elements are both required for follicle-stimulating hormone-induced transferrin promoter activation in Sertoli cells. *Endocrinology* 140:1262–1271.
- Chaudhary, J., P. D. Whaley, A. Cupp, and M. K. Skinner. 1996. Transcriptional regulation of Sertoli cell differentiation by follicle-stimulating hormone at the level of the *c-fos* and transferrin promoters. *Biol. Reprod.* 54:692–699.
- Data Desk4. 1994. A New Power of Statistical Vision. Data Descriptions Inc., Ithaca, NY.
- Davis, R. J., and M. P. Czech. 1986. Regulation of transferrin receptor expression at the cell surface by insulin-like growth factors, epidermal growth factor and platelet-derived growth factor. *EMBO J.* 5:653–658.
- Farrant, J. L. 1954. An electron microscopic study of ferritin. *Biochim. Biophys. Acta* 13:569–576.
- FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.
- Ford, J. J., T. H. Wise, and D. D. Lunstra. 1997. Negative relationship between blood concentrations of follicle-stimulating hormone and testicular size in mature boars. *J. Anim. Sci.* 75:790–795.
- Ford, J. J., T. H. Wise, D. D. Lunstra, and G. A. Rohrer. 2001. Interrelationships of porcine X and Y chromosomes with pituitary gonadotropins and testicular size. *Biol. Reprod.* 65:906–912.
- Hall, S. H., M.-C. Berthelon, O. Avallet, and J. M. Saez. 1991. Regulation of *c-fos*, *c-jun*, *jun-B*, and *c-myc* messenger ribonucleic acids by gonadotropin and growth factors in cultured pig Leydig cell. *Endocrinology* 129:1243–1249.
- Harrison, P. M., and P. Arosio. 1996. The ferritins: Molecular properties, iron storage function and cellular regulation. *Biochim. Biophys. Acta* 1275:161–203.
- Harrison, P. M., G. A. Clegg, and K. May. 1980. Ferritin structure and function. Pages 131–171 in *Iron in Biochemistry and Medicine*. Vol. 2. A. Jacobs and M. Worwood, ed. Academic Press, London.
- Huang, Y.-T., and R. K. Johnson. 1996. Effect of selection for size of testes in boars on semen and testis traits. *J. Anim. Sci.* 74:750–760.
- Huggenvik, J. I., R. L. Idzerda, L. Haywood, D. C. Lee, G. S. McKnight, and M. D. Griswold. 1987. Transferrin messenger ribonucleic acid: molecular cloning and hormonal regulation in rat Sertoli cells. *Endocrinology* 120:332–340.
- Iancu, T. C. 1992. Ferritin and hemosiderin in pathological tissues. *Electron. Microsc. Rev.* 5:209–229.
- Kelly, T. M., C. Q. Edwards, A. W. Meikle, and J. P. Kushner. 1984. Hypogonadism in hemochromatosis: Reversal with iron depletion. *Ann. Intern. Med.* 101:629–632.
- Lim, K., and B. D. Hwang. 1995. Follicle-stimulating hormone transiently induces expression of protooncogene *c-myc* in primary Sertoli cell cultures of early pubertal and prepubertal rat. *Mol. Cell. Endocrinol.* 111:51–56.
- Lin, T., J. Blaisdell, K. W. Barbour, and E. A. Thompson. 1988. Transient activation of *c-myc* protooncogene expression in Leydig cells by human chorionic gonadotropin. *Biochem. Biophys. Res. Commun.* 157:121–126.
- Lucesoli, F., M. Caligiuri, M. F. Roberti, J. C. Perazzo, and C. G. Fraga. 1999. Dose-dependent increase of oxidative damage in the testes of rats subjected to acute iron overload. *Arch. Biochem. Biophys.* 372:37–43.
- Lunstra, D. D., J. J. Ford, R. K. Christenson, and R. D. Allrich. 1986. Changes in Leydig cell ultrastructure and function during pubertal development in the boar. *Biol. Reprod.* 34:145–158.
- Lunstra, D. D., J. J. Ford, J. Klindt, and T. H. Wise. 1997. Physiology of the Meishan boar. *J. Reprod. Fertil. (Suppl.)* 52:181–193.
- Markwell, M. K., S. M. Haas, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* 87:206–210.
- May, M. E., and W. W. Fish. 1977. The isolation and properties of porcine ferritin and apoferritin. *Arch. Biochem. Biophys.* 182:396–403.
- Mazur, A., and E. Shorr. 1950. A quantitative immunochemical study of ferritin and its relation to the hepatic vasodepressor material. *J. Biol. Chem.* 182:607–627.
- Merker, H. J., J. Vormann, and T. Gunther. 1996. Iron-induced injury of rat testis. *Andrologia* 28:267–273.
- Okwun, O. E., G. Igboeli, D. D. Lunstra, J. J. Ford, and L. Johnson. 1996. Testicular composition, number of A spermatogonia, germ cell ratios, and number of spermatids in three different breeds of boars. *J. Androl.* 17:301–309.
- Palencia-Dominguez, T., J. M. Martinez-Cervera, and X. Fuentes-Arderiu. 1997. Evaluation of a new measurement procedure for the concentration of ferritin in serum. *Eur. J. Clin. Chem. Biochem.* 35:117–120.
- Papadimas, J., E. Mandala, G. Pados, B. Kokkas, G. Georgiadis, B. Tarlatzis, J. Bontis, Z. Sinakos, and S. Mantalenakis. 1996.

- Pituitary-testicular axis in men with  $\beta$ -thalassaemia major. *Hum. Reprod.* 11:1900–1904.
- Perls, M. 1867. Nachweis von Eisen oxyd in gewissen pigmenten. *Virchows Arch.* 39:42.
- Picard, V., G. Govoni, N. Jabado, and P. Gros. 2000. Nramp 2 (DCT1/DMT1) expressed at the plasma membrane transports iron and other divalent cations into a calcein-accessible cytoplasmic pool. *J. Biol. Chem.* 275:35738–35745.
- Rathje, T. A., R. K. Johnson, and D. D. Lunstra. 1995. Sperm production in boars after nine generations of selection for increased weight of testis. *J. Anim. Sci.* 73:2177–2185.
- Roberts, K. P., and M. D. Griswold. 1990. Characterization of rat transferrin receptor cDNA: The regulation of transferrin receptor mRNA in testes and in Sertoli cells in culture. *Mol. Endocrinol.* 4:531–542.
- Rohrer, G. A., J. J. Ford, T. H. Wise, J. L. Vallet, and R. K. Christenson. 1999. Identification of quantitative trait loci affecting female reproductive traits in a multigeneration Meishan-White composite swine population. *J. Anim. Sci.* 77:1385–1391.
- Rohrer, G. A., T. H. Wise, D. D. Lunstra, and J. J. Ford. 2001. Identification of genomic regions controlling plasma FSH concentrations in Meishan-White Composite boars. *Physiol. Genomics* 6:145–151.
- Russell, L. D., J. Warren, L. Debeljuk, L. L. Richardson, P. L. Mahar, K. G. Waymire, S. P. Amy, A. J. Ross, and G. R. MacGregor. 2001. Spermatogenesis in *Belw*-deficient mice. *Biol. Reprod.* 65:318–332.
- Skinner, M. K., and M. D. Griswold. 1982. Secretion of testicular transferrin by cultured Sertoli cells is regulated by hormones and retinoids. *Biol. Reprod.* 27:211–221.
- Tiffany, T. O., and C. A. Burtis. 1987. Fluorometry, nephelometry and turbidimetry. Pages 74–77 in *Fundamentals of Clinical Chemistry*. 3rd ed. N. W. Tietz, ed. W.B. Saunders Co., Philadelphia, PA.
- Toebosch, A. M., M. J. Kroos, and J. A. Grootegoed. 1987. Transport of transferrin-bound iron into rat Sertoli cells and spermatids. *Int. J. Androl.* 10:753–764.
- Wauben-Penris, P. J. J., G. J. Strous, and H. A. van der Donk. 1988. Kinetics of transferrin endocytosis and iron uptake by intact isolated rat seminiferous tubules and Sertoli cells in culture. *Biol. Reprod.* 38:853–861.
- Wise, T., D. D. Lunstra, and J. J. Ford. 1996. Differential pituitary and gonadal function of Chinese Meishan and European white composite boars: Effects of gonadotropin-releasing hormone stimulation, castration, and steroidal feedback. *Biol. Reprod.* 54:146–153.
- Wise, T., D. Lunstra, and J. J. Ford. 1999. Relationships of FSH concentrations and testicular weight with sperm production and testicular ferritin and iron concentrations. Page 72 in *Proc. 15th NIH Testis Workshop*, Louisville, KY.
- Wise, T., E. L. Zanella, D. D. Lunstra, and J. J. Ford. 2000. Relationships of gonadotropins, testosterone, and cortisol in response to GnRH and GnRH antagonist in boars selected for high and low follicle-stimulating hormone levels. *J. Anim. Sci.* 78:1577–1590.
- Worwood, M., W. Aherne, S. Dawkins, and A. Jacobs. 1975. The characteristics of ferritin from human tissues, serum and blood cells. *Clin. Sci. Mol. Med.* 48:441–451.
- Wu, K.-J., A. Polack, and R. Dalla-Favera. 1999. Coordinated regulation of iron-controlling genes, H-ferritin and *IRP2*, by c-MYC. *Science (Wash. DC)* 283:676–679.

**References**

This article cites 40 articles, 21 of which you can access for free at:  
<http://jas.fass.org/cgi/content/full/81/2/503#BIBL>

**Citations**

This article has been cited by 1 HighWire-hosted articles:  
<http://jas.fass.org/cgi/content/full/81/2/503#otherarticles>